PATENT
Attorney Docket No. 3495.0111-10

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Bernard DUJON et al.

Serial No.: 09/244,130

Filed: February 4, 1999

Group Art Unit: 1633

Examiner: KAUSHAL, S.

For: NUCLEOTIDE SEQUENCE ENCODING THE ENZYME I-SCEI AND THE USES THEREOF

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

DECLARATION OF ANDRE CHOULIKA

I, Andre Choulika, declare that:

1. I have read and understood application Serial No. 09/244,130, including the pending claims, and on information and belief copies are attached hereto as Exhibit 1;

2. I am an inventor of the subject matter claimed in application Serial No. 09/244,130;

3. On page 38, application Serial No. 09/244,130 discloses transgenic cell line D3 containing the I-*Sce*I site at undetermined locations in the genome;

4. On page 38, application Serial No. 09/244,130 discloses that D3 cells are ES cells able to generate transgenic animals;

5. On page 47-48, application Serial No. 09/244,130 discloses clone MLOP014, a Ψ2 mouse cell line transfected with pMLV LTR SAPLZ containing the I-*Sce*I site and selected for phleomycin resistance. Figure 13 of application Serial No. 09/244,130 schematically depicts pMLV LTR SAPLZ, which contains an I-*Sce*I site;

6. Transgenic mice were generated from the D3 cells disclosed in application Serial No. 09/244,130;

7. Details concerning the generation of transgenic mice containing the I-*Sce*I recognition and cleavage site are as follows:

Ψ2MLOP014 defective recombinant retrovirus producer cell lines were used to infect 5×10^6 D3 cells. After infection, D3 cells were plated in fresh mouse embryonic fibroblast (MEF) treated gelatin coated plates for 48 hours. 48 hours post-infection, D3 cells were selected in an ES cell culture medium with 10μg/ml of phleomycin. Fresh phleomycin was supplied every 48 hours. 10^4 new mitomycin C treated MEFs were supplied every 4 days. 12 days post-selection, 12 phleomycin-resistant D3 clones were isolated and analyzed for PhleoLacZ expression by X-gal staining, and the highest ß-galactosidase-expressing clones were selected. Genomic DNA of D3 infected clones was analyzed by southern blot hybridization by *Pst*I, *Eco*RV, and I-*Sce*I digestion. Three clones were selected: D3-MLOP.a, D3-MLOP.j, and D3-MLOP.5. 9-12 D3 cells of each clone expressing ß-galactosidase were injected into the blastocel of the blastocyst from mouse strain 129. 12 injected blastocysts were reimplanted into 4 month old DBA2 foster mother mice (6 per uterine duct) and allowed to develop to term;

8. Three offspring resulting from the injection of D3-MLOP.5 cells in blastocysts were chimeric at various percentages ranging from 50% to 85% according to hair phenotyping;

9. All three offspring contained integrated I-*Sce*I sites;

10. Exhibit 2 depicts southern blot hybridization of genomic DNA of D3 infected clones;

11. Exhibit 3 depicts D3 ES cells containing I-*Sce*I site;

12. Exhibit 4 depicts transgenic mice containing I-*Sce*I sites, which were obtained from D3 cells.

13. The undersigned further declares that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing therefrom.

Dated: Oct. 9/Oct/2001

By: _____
Andre Choulika